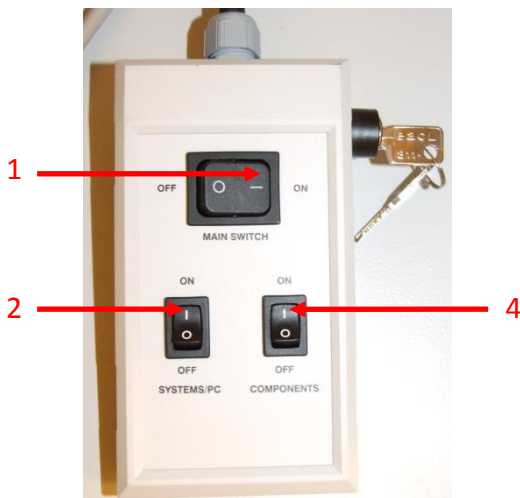


ZEISS LSM 780 CONFOCAL MICROSCOPE USER MANUAL

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START THE SYSTEM

1. Press « MAIN SWITCH » button.
2. Press « SYSTEM PC » button.
3. Switch on the PC and log in the « USER » session.
4. When Windows has started, press « COMPONENT » button.
5. Switch on the fluorescent light.

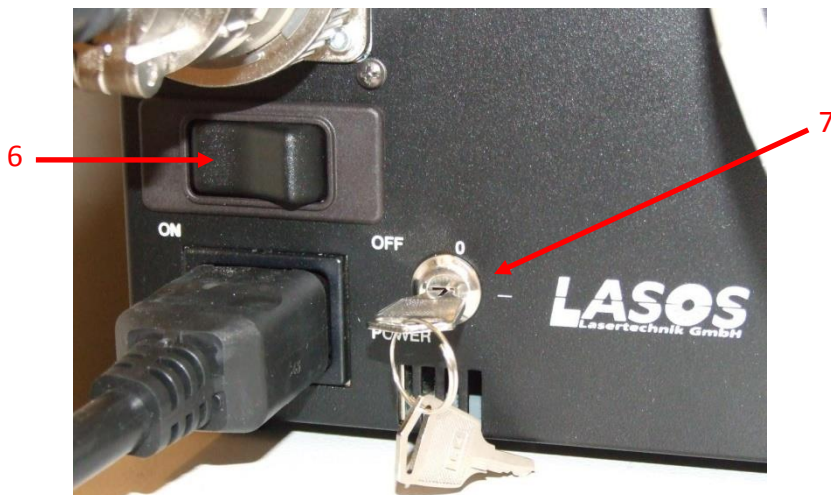


On the argon laser power supply box :

6. Check if the switch is on « ON » position.
7. Turn the *Power* key on « I ».

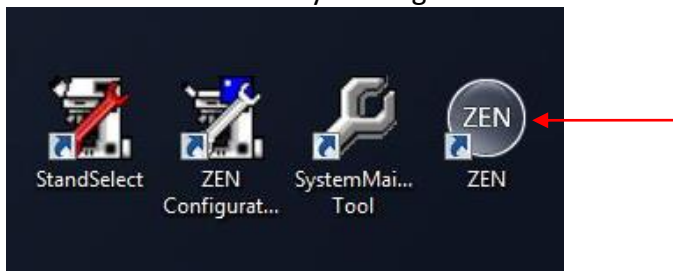
On the small laser box:

8. Push the button up to « LASER RUN ».
9. Once the green LED is lit (about 5 minutes after step 7) turn the knob to the 9 o'clock position, while taking care of not switching on the red LED.



START ZEN SOFTWARE

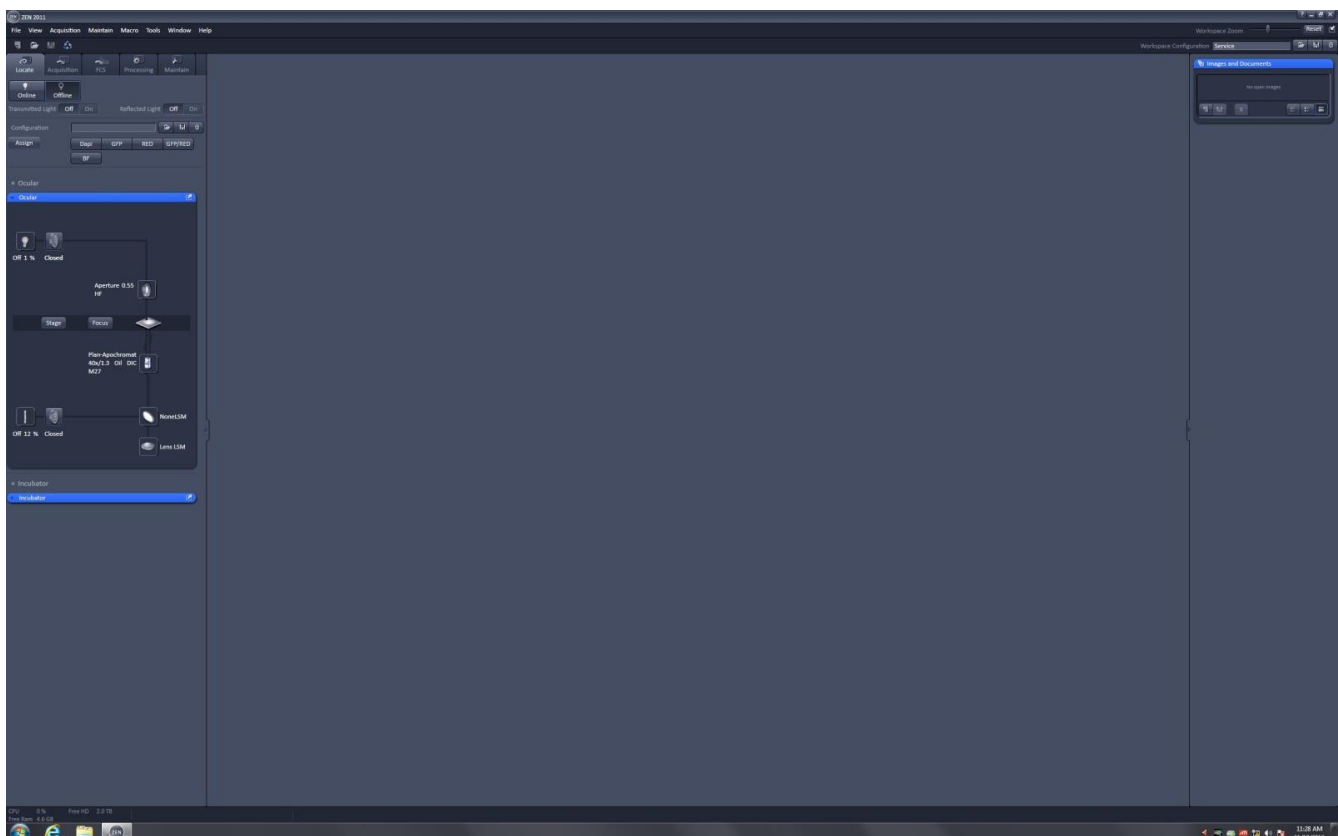
1. Start the software by clicking on ZEN 2012 icon on window desktop.



2. Click on the START SYSTEM button to have access to the acquisition menu.



3. Zen software is divided in 3 parts: the left side is dedicated to acquisition settings, the central part displays your image and the right side shows you all images that you've recorded.

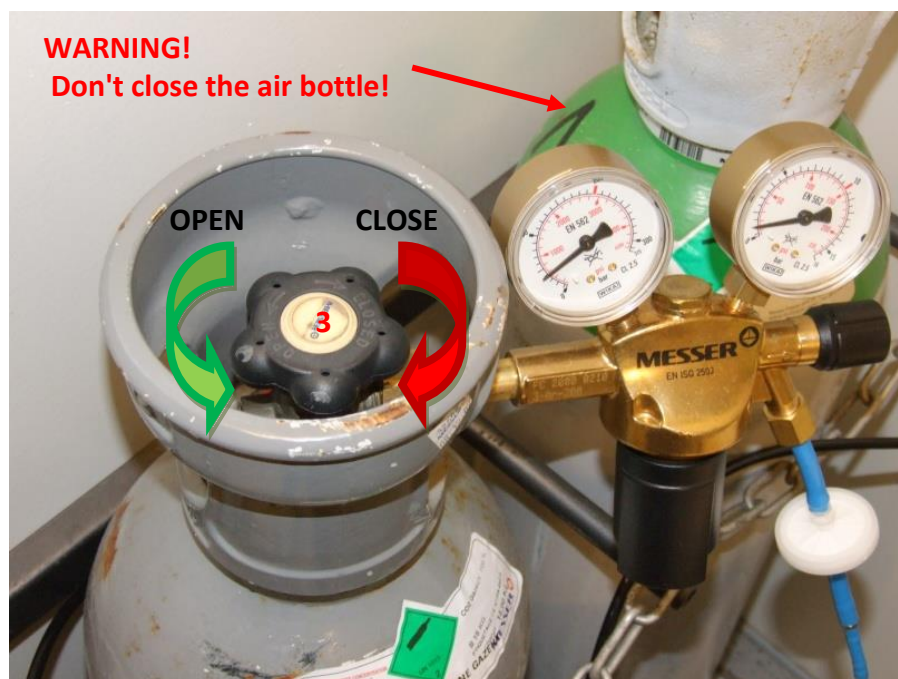
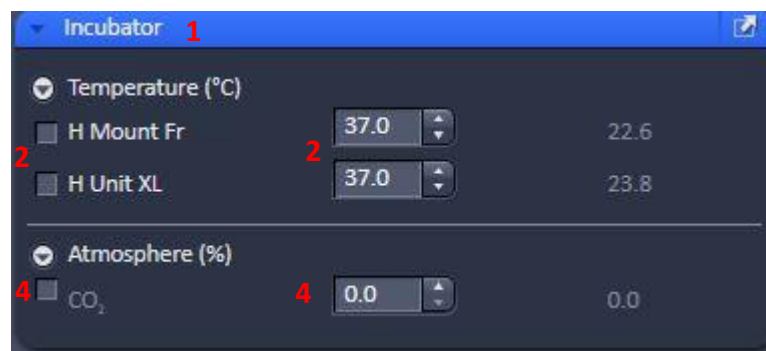


SET THE TEMPERATURE AND THE CO₂ CONTROLLERS

1. Open the INCUBATOR window.
2. Enter the temperature value that you need and tick the box.
3. Open the CO₂ bottle.
4. Enter the percentage of CO₂ and tick the box.

Warning !!!

At the end of your session, please untick all boxes and close the CO₂ bottle.

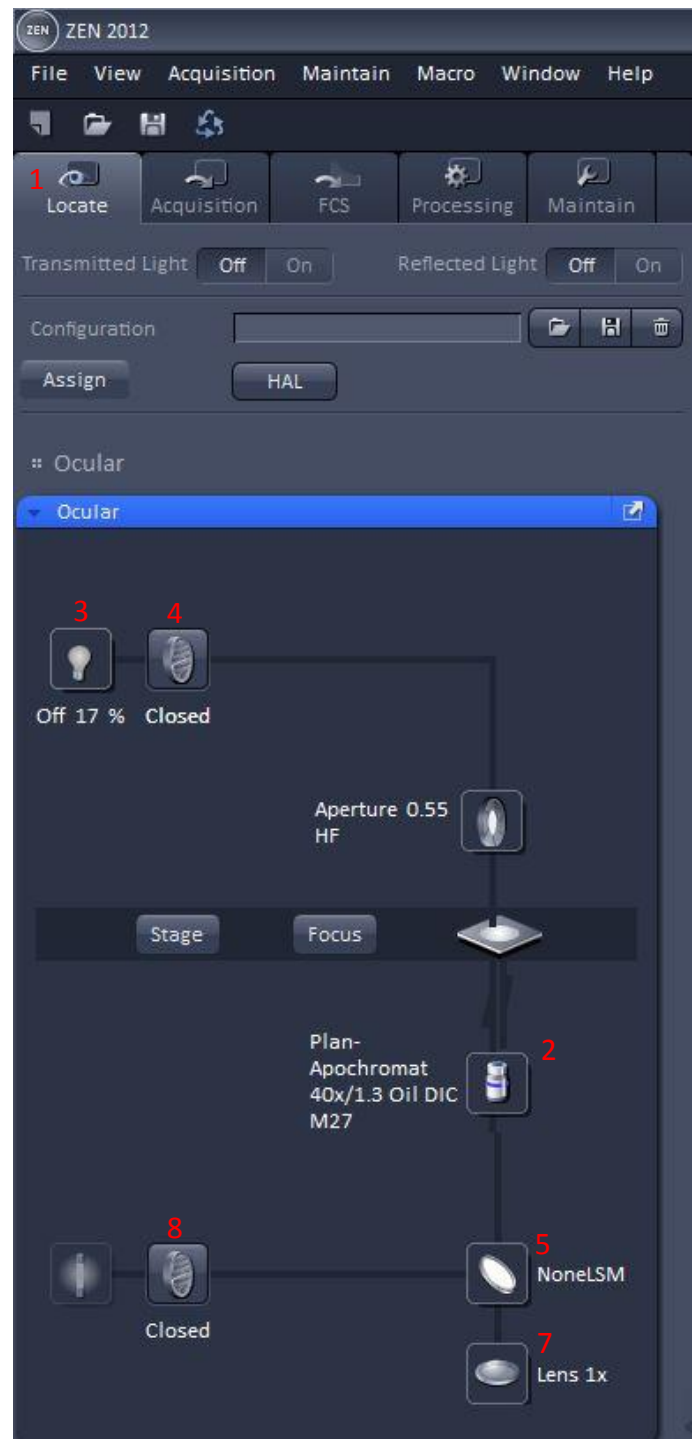


OCULARS OBSERVATION

1. Select the LOCATE tab (first on the left).
2. Choose your objective.

Depending on the type of illumination that you would like to use, please follow these different steps :

3. Light on/light off/choose the intensity of transmission light source
4. Open/Close the transmission shutter
5. Select the "NoneLSM" filter
6. Open the fluorescent lamp shutter; a little blue LED lights up
7. Select the corresponding filter for your fluorescence
8. Open/Close the fluorescence shutter
9. Adjust the fluorescent lamp intensity



MICROSCOPE STAND PRESENTATION

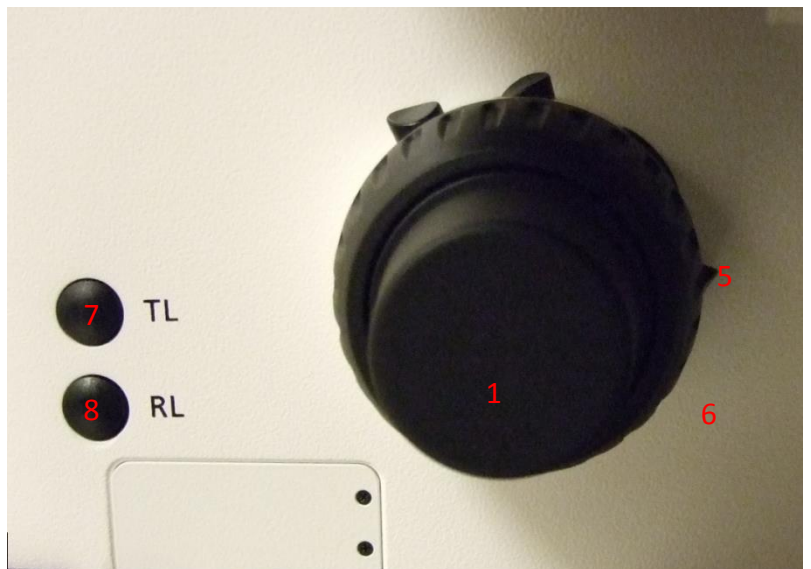
On the left side :

1. Focus adjustment knob
2. no function
3. no function
4. Optovar 1x
5. Previous fluorescence filter
6. Next fluorescence filter



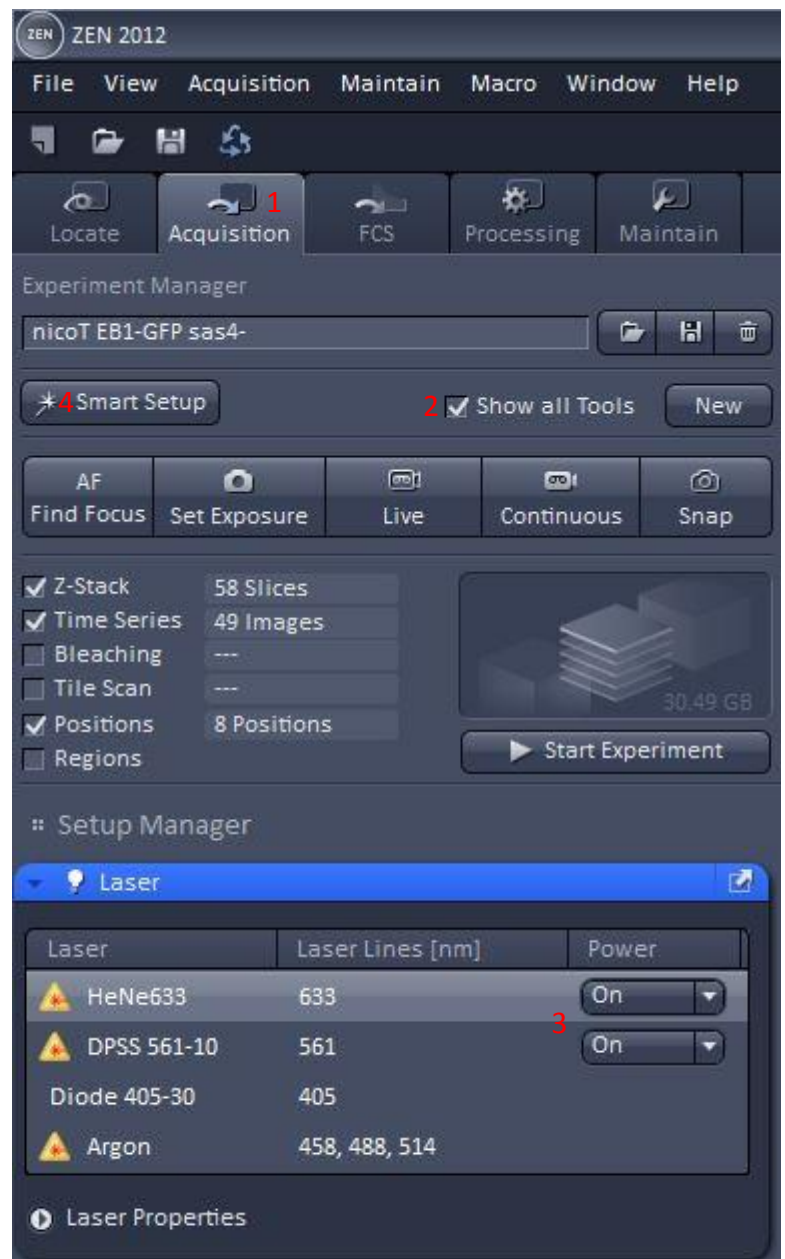
On the right side :

1. Focus adjustment knob
2. Lowers the objective to its lower position
3. Rises the objective to its working position
4. no function
5. Switch to an objective with a lower magnification
6. Switch to an objective with a higher magnification
7. Open/close the transmitted light shutter
8. Open/close the fluorescent lamp shutter

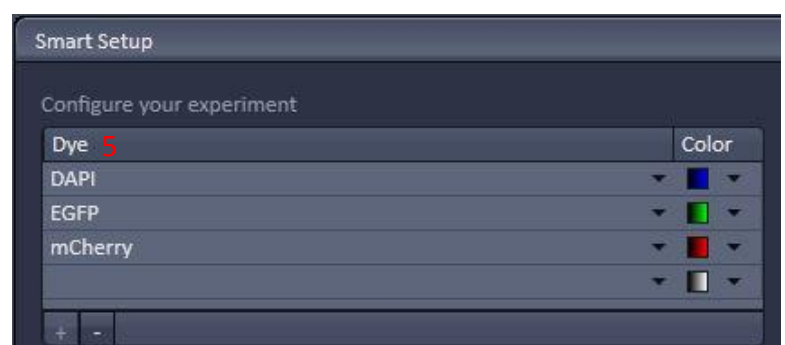


ACQUIRE ONE OR SEVERAL COLORS (STAINING)

1. Select the ACQUISITION tab
2. Tick SHOW ALL TOOLS checkbox
3. In the LASER tool box activate the required lasers (power on)
4. Open SMART SETUP
5. Then in the DYE menu, select the different dyes and their respective colors.



- 6.



6. Several methods are suggested :

-FASTEST : allows simultaneous acquisition for all your channels ; this is the fastest method, but cross talk between consecutive channels can be important.

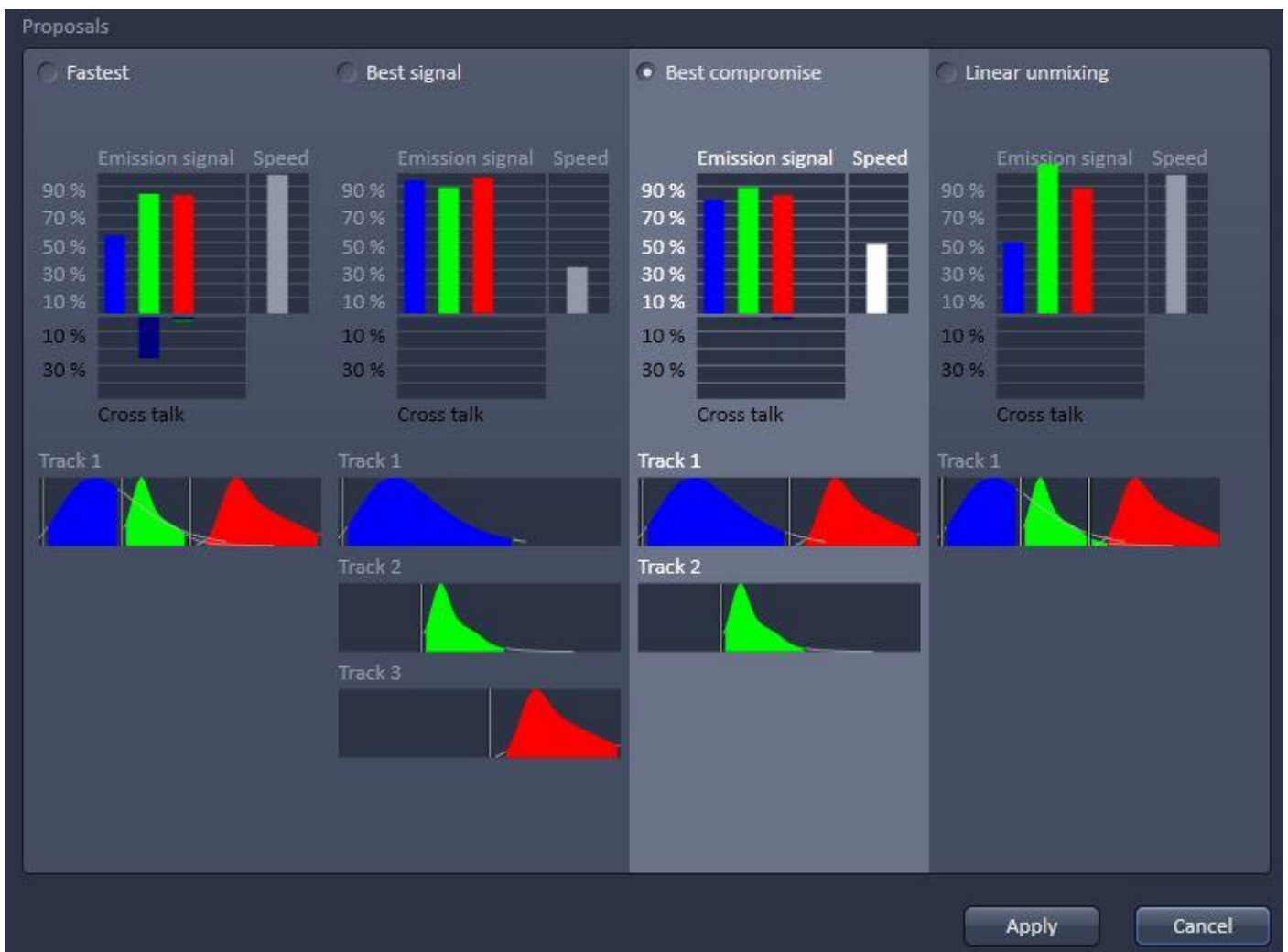
-BEST SIGNAL : each channel is acquired separately. This method is the slowest one but it avoids (as far as it is possible) cross talk between consecutive channels.

-BEST COMPROMISE : compromise between speed and reduction of cross talk.

Example : both blue and red channels are acquired simultaneously, then the green one is acquired separately.

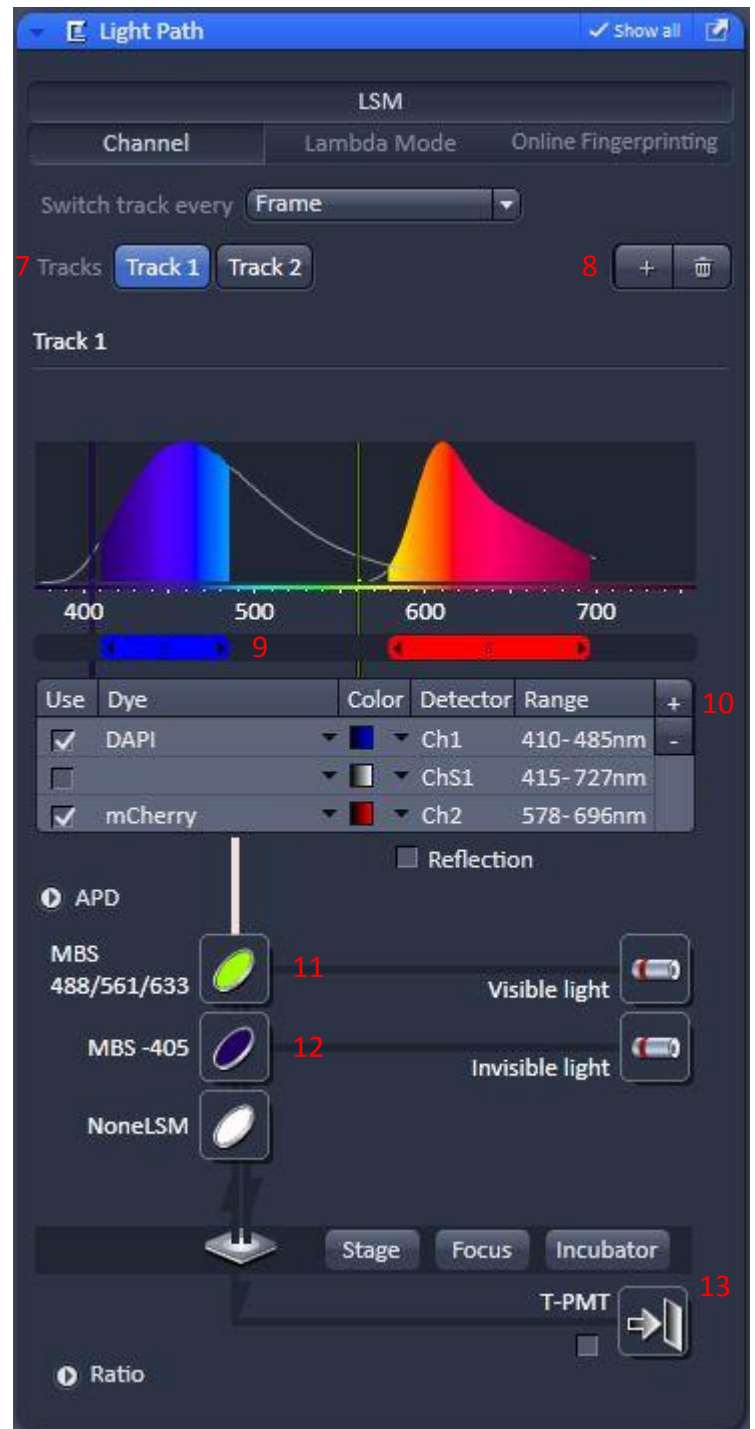
-LINEAR UNMIXING : use spectral capacity of the system to separate different dyes simultaneously. Beforehand, you need to acquire samples which are stained with only one dye.

Remarks : These modes are just a base to start a configuration. The software doesn't necessarily choose the best one . You have to check by yourself and then modify appropriately the settings .

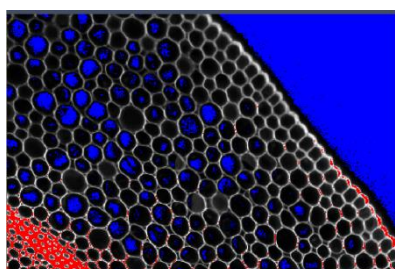


7. In the LIGHT PATH tool box, each TRACK corresponds to one sequence of one or several colors.
8. Add or remove sequences by clicking on +/-
9. Adapt wavelength detection by adjusting the detection window for each channel.
10. Add or remove channels with +/- for each sequence.
11. Select, if possible, the same dichroic for each sequence in order to accelerate the change between sequences.
If no dichroics is required, select the "plate" filter.
12. Activate the 405 dichroic for each sequence even if you don't need it.
13. Tick T-PMT to acquire transmission images in one of your fluorescence sequences.

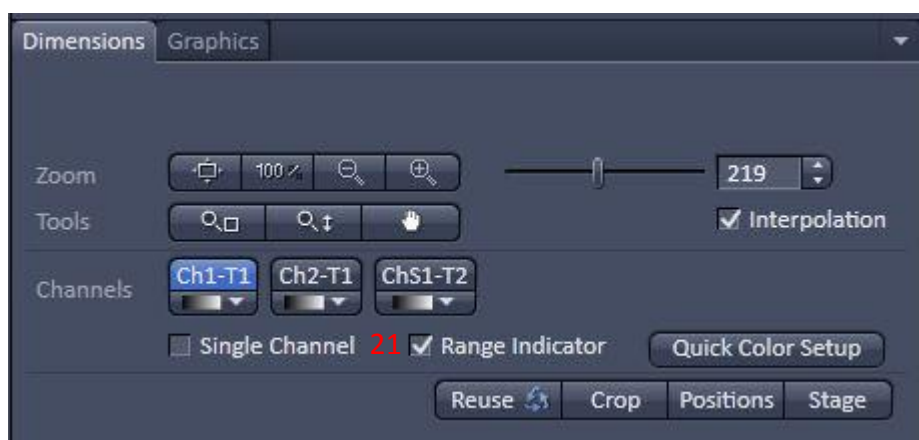
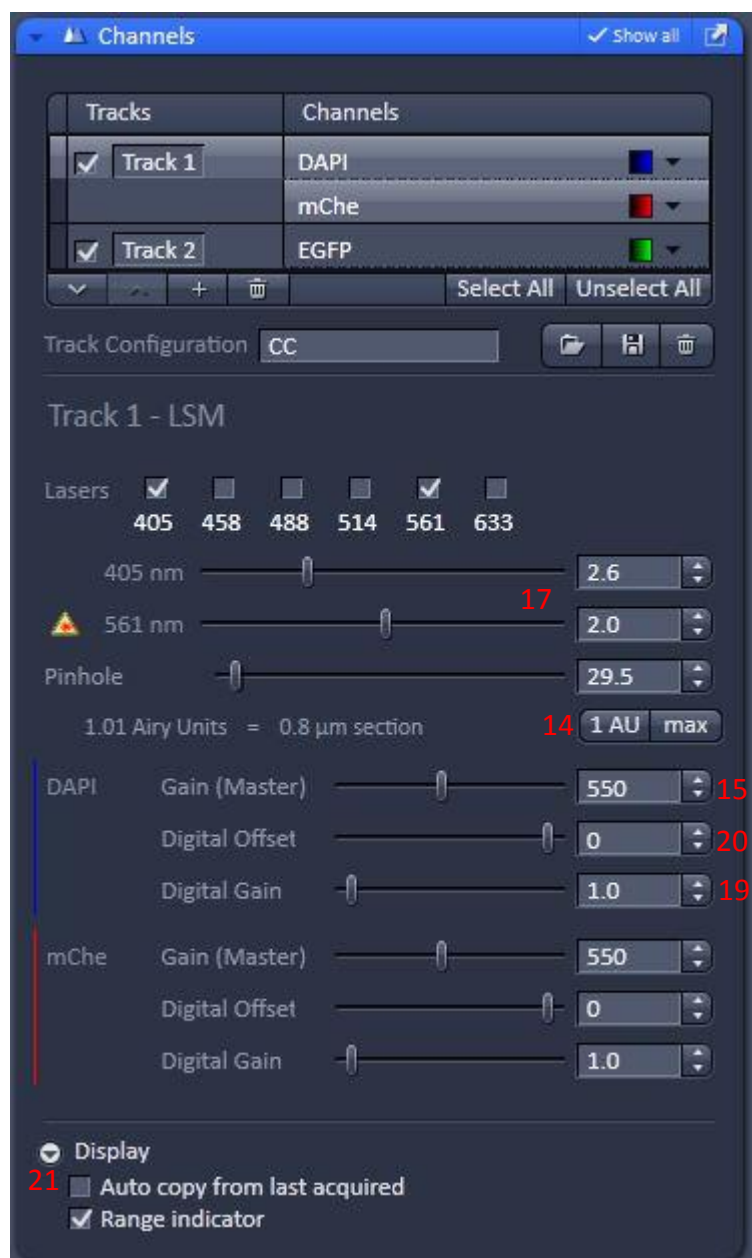
14.



14. In the CHANNELS tool box, adjust the pinhole at 1 AU (Airy unit) for each channel in order to obtain the best resolution/signal ratio.
15. Fix the GAIN (MASTER) at 550 for each channel except for T-PMT.
16. Click on LIVE to change your parameters if necessary.
17. Adjust the laser power for each channel.
18. You can increase the GAIN (MASTER) to decrease the laser power if it is too high. By increasing too much the gain, the signal/noise ratio will be impacted.
19. Don't increase the DIGITAL GAIN except if the GAIN (MASTER) is high enough.
20. Adjust the DIGITAL OFFSET in order to set background to 0.
21. To find adapted parameters for gain and laser power, it is recommended to use the « Range Indicator » display. In this representation, saturated pixels are red and black pixels are blue.



22.



22. In the ACQUISITION MODE tool box choose the sampling of your image which has an impact on the resolution. See p12

23. Move in Z to find the plane of interest thanks to knobs on each side of the microscope.

24. Choose the scan speed (faster it is, worse is the signal/noise ratio).

25. Average the image (AVERAGING) if the signal/noise ratio is not satisfying. If you have a fixed sample the Frame mode is more appropriate. If you have a living sample the Line mode is better. (Method Mean is recommended)

26. Bidirectional mode allows you to acquire 2 time faster. This mode must be well corrected with a high contrasted sample, phase settings (Corr X) are then quite important.

27. Zoom in (be careful on photobleaching and image sampling, see p12).

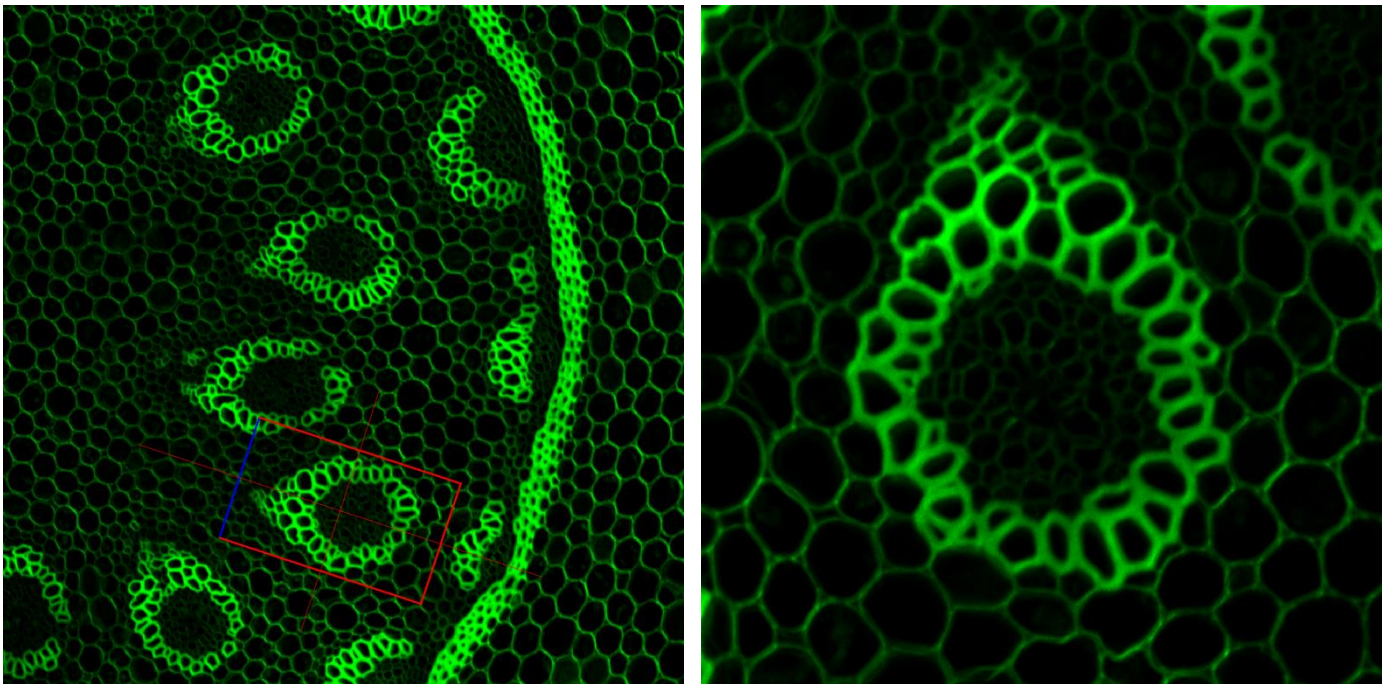
28. If necessary, move your zoom area to see your object of interest.

29. If necessary, rotate your image.

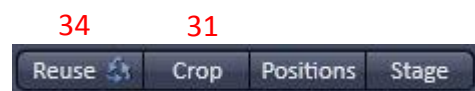
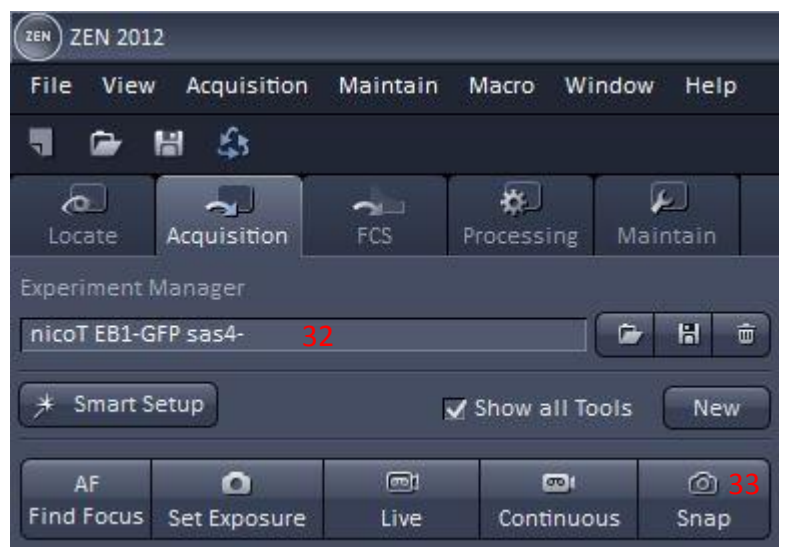
30. You can make all of these actions by moving the square zone which represents your final image.



31. You can directly zoom in the region of interest with the CROP option, just under the image. Click on CROP, a framework appear on the image. The blue line represents the top of the final image. You can translate, rotate, make bigger or smaller the framework. Then click on LIVE or SNAP.



32. Save your configuration.
33. Click on SNAP to acquire an image.
34. You can reuse the parameters of an image previously acquired by selecting the image then by clicking on REUSE.



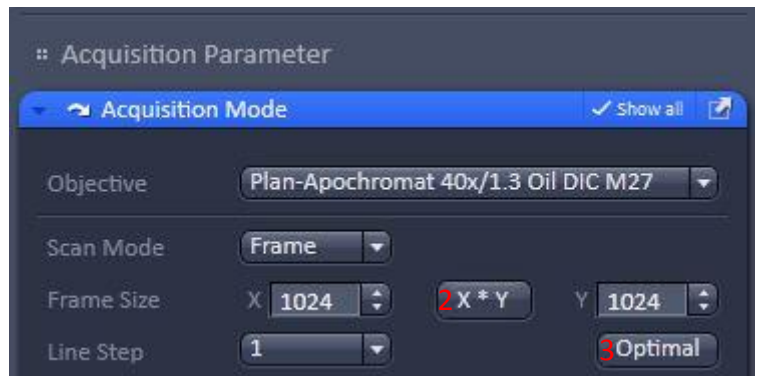
SPATIAL SAMPLING (Image resolution)

In order to obtain the optimal resolution for your image, the image voxel size must be equal to the half of the resolution of the objective that you use (Nyquist criteria). On a light microscope, the lateral resolution (in XY) is better than the axial resolution (in Z).

- Lateral resolution :

Choose the pixel size by modifying

1. the zoom : higher is the zoom, smaller is the image pixel size (page 10-11).
2. the frame size (number of pixels composing your image).
3. To choose automatically the best resolution depending on the objective and the zoom, click on OPTIMAL.



Setting pixel size smaller than the resolution is useless (over-sampling).

However you can under-sample the image in order to increase the speed of the acquisition and decrease the loss of fluorescence because of the photobleaching effect.

- Axial resolution :

The axial resolution is inversely proportional to the pinhole size. To obtain the best resolution with your objective, you have to work with a pinhole adjusted at 1 Airy Unit size (click on 1AU in the CHANNELS tool box).

4. To obtain the optimal sampling in Z, depending on the pinhole click on SMALLEST.
5. For an acquisition with a higher interval, choose manually the value in INTERVAL.

If a high axial resolution is not necessary, you can acquire with a higher interval in Z in order to increase the speed of acquisition and decrease the loss of fluorescence because of the photobleaching effect.



ACQUIRE A Z SERIES

1. Proceed in the same manner than in the previous section ACQUIRE ONE OR SEVERAL COLORS to fix the settings. Be careful, you must choose your parameters in the brightest plane .

2. Tick Z-STACK and in MULTIDIMENSIONAL ACQUISITION rubric open the Z-Stack menu.

Two methods :

If you know how thick is your sample

3. Choose the tab CENTER.

4. Make the focus on the center of the sample then click on CENTER.

If you don't know how thick is your sample

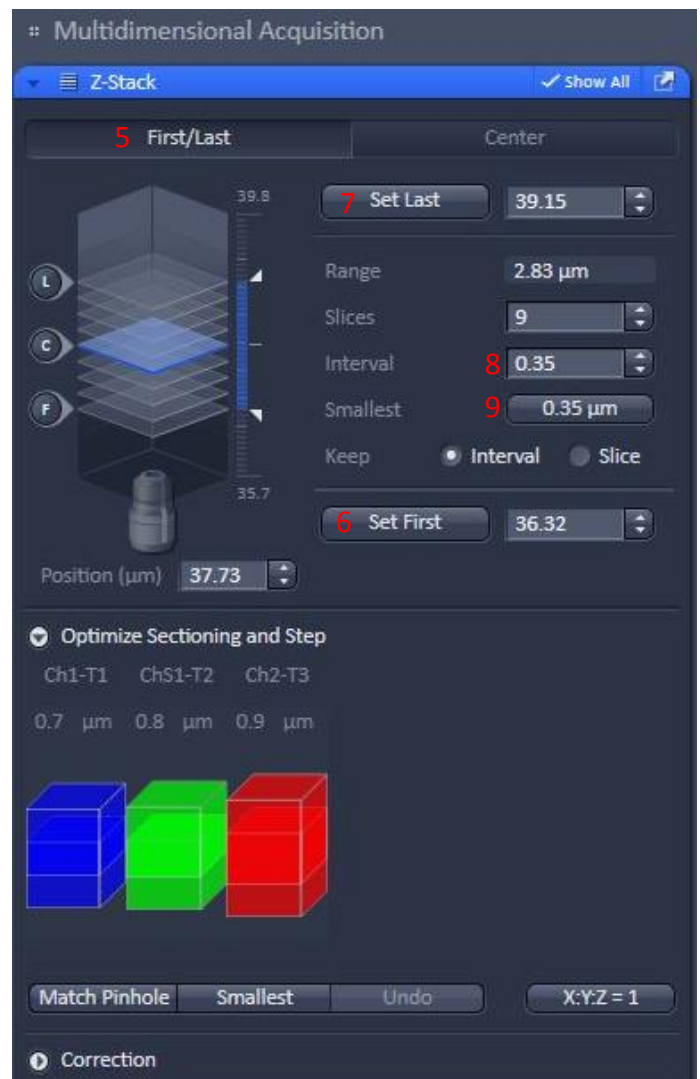
5. Choose the tab FIRST/LAST.

6. Make the focus on the bottom (coverslip side) then click on SET FIRST.

7. Make the focus on the top (slide side) then click on SET LAST.

8. If you tick INTERVAL, you can choose the step between two sections by setting manually the value in INTERVAL (μm).

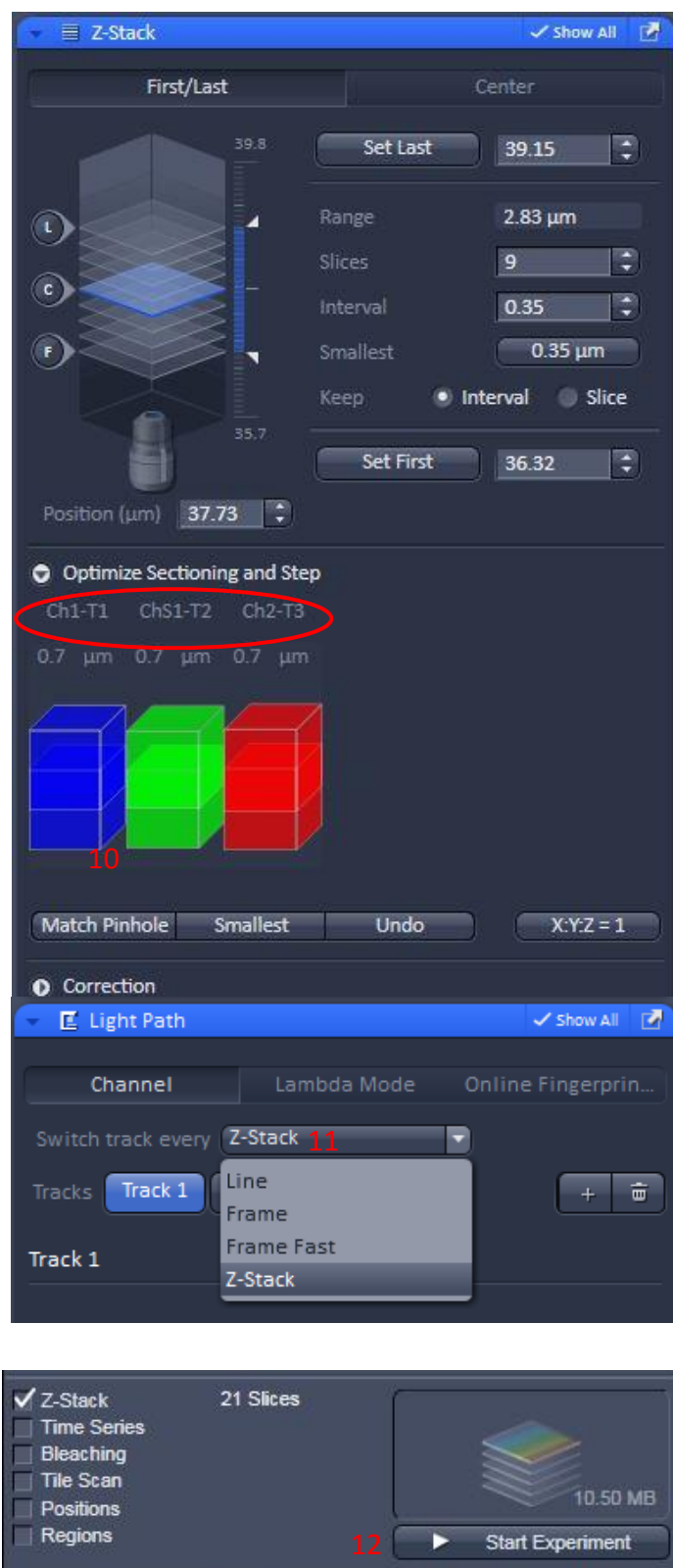
9. You can click on SMALLEST to automatically choose the best resolution depending on the pinhole size (beforehand click on 1AU in the CHANNELS tool box).



10. If you want to make co-localisation analysis, click on MATCH PINHOLE in order to automatically adjust pinholes sizes and to obtain the same voxel size for all channels.

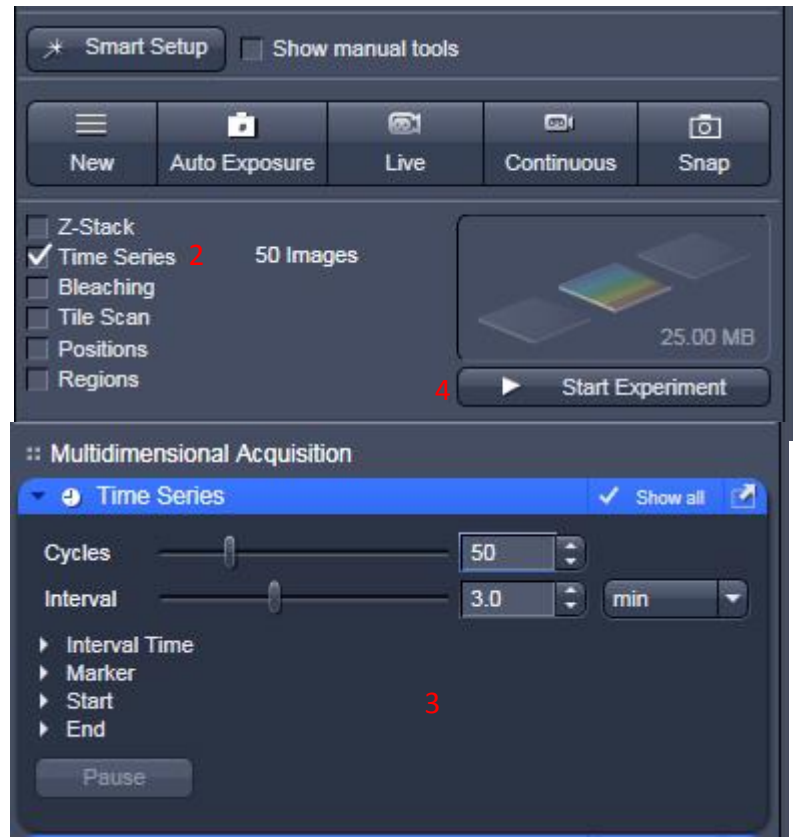
11. In LIGHT PATH go to SWITCH TRACK EVERY and choose Z-STACK if you want to acquire an entire stack channel by channel. Otherwise, choose FRAME to acquire all channels per frame.

12. Click on « Start Experiment » to start the acquisition.



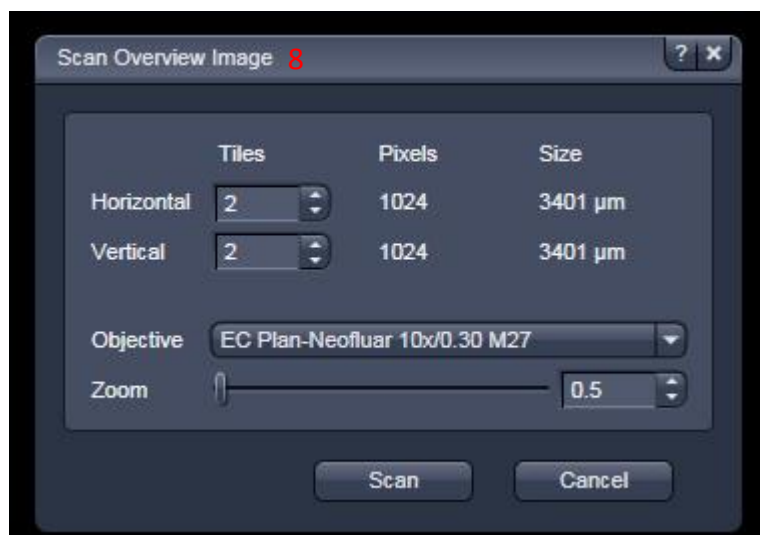
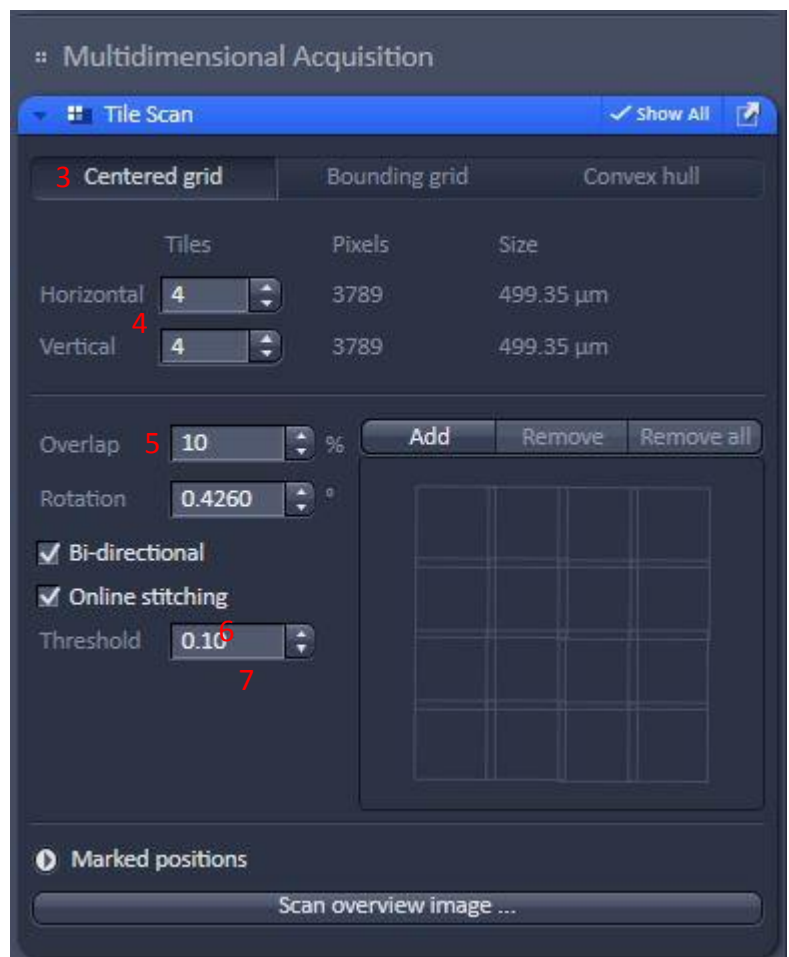
ACQUIRE A TIME SERIES

1. Proceed in the same way than in the previous section ACQUIRE ONE OR SEVERAL COLORS to fix the settings.
2. Tick TIME SERIES to open the tool box in the MULTIDIMENSIONAL ACQUISITION menu.
3. Choose the number of cycle and the interval between each time point.
4. Click on START EXPERIMENT to start the acquisition



ACQUIRE A MOSAIC IMAGE

1. Proceed in the same way than in the previous section ACQUIRE ONE OR SEVERAL COLORS to fix the settings.
2. Tick TILE SCAN to open the tool box in the menu MULTIDIMENSIONAL ACQUISITION.
3. There are different modes in TILE SCAN. Go to CENTERED GRID to acquire a mosaic from the center.
4. Choose the number of lines and columns.
5. Let OVERLAP at 10%.
6. Tick BI-DIRECTIONAL to go faster.
7. Tick ONLINE STICHING and let the THRESHOLD at 0.10, except if you make time lapse tilescan imaging or multiple positions tilescan imlaging.
8. You can have an overview of your mosaic by clicking on SCAN OVERVIEW IMAGE. You can choose an objective and a zoom different from the final acquisition (be careful when you change from an oil objective to a dry one or inversely).
9. Click on START EXPERIMENT to start the acquisition.

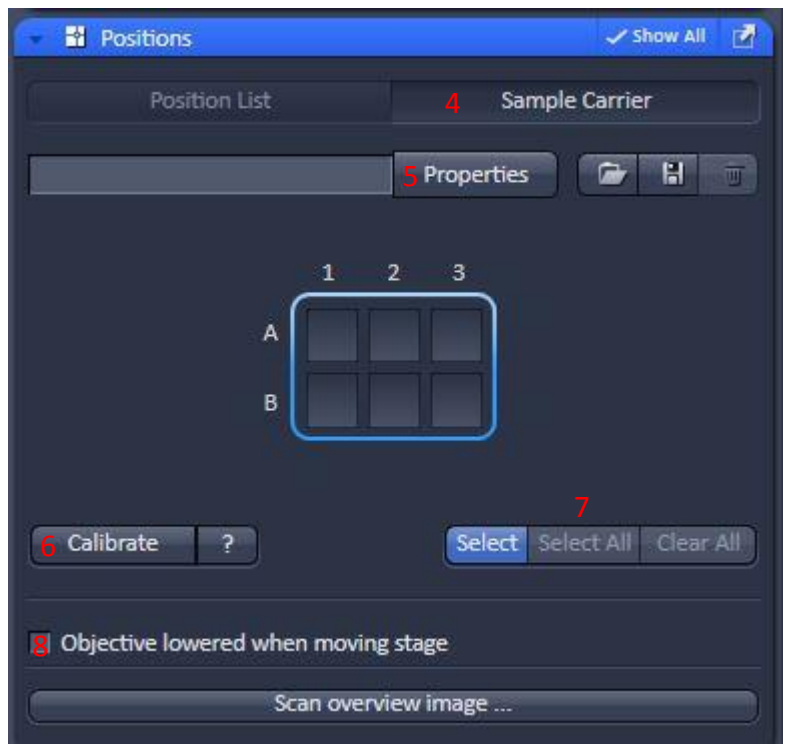
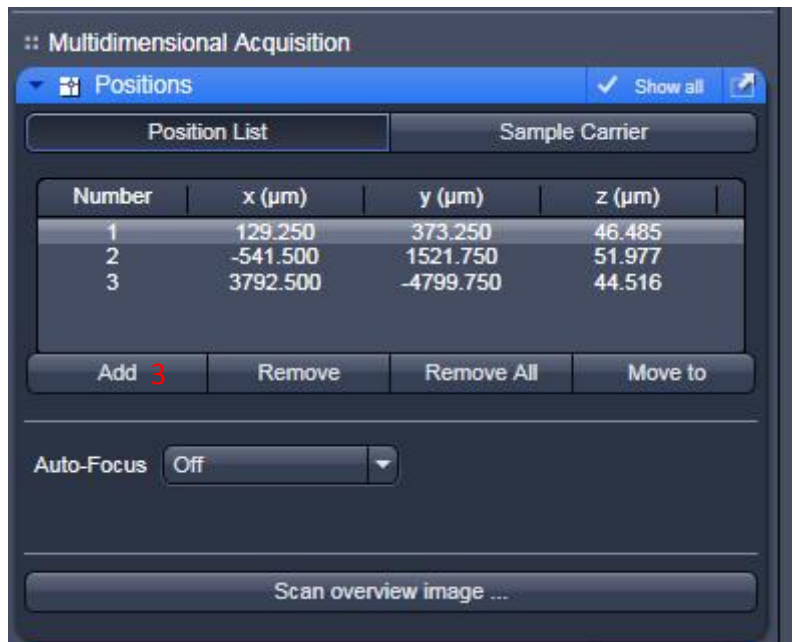
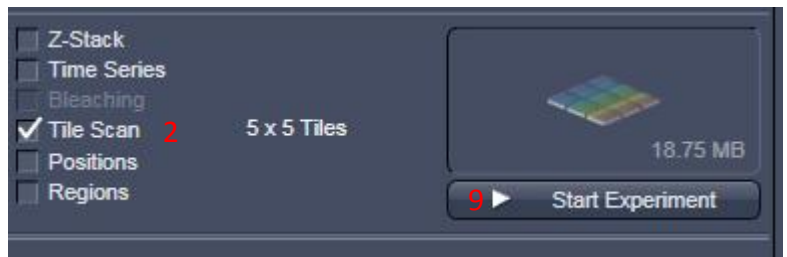


10. To make a mosaic image including different fields of interest, go to the option BOUNDING GRID.
11. Look for your fields of interest in LIVE and save their coordinates by clicking on ADD. To remove a position, select the coordinates in the list and click on REMOVE. To remove all, click on REMOVE ALL.
12. Dimensions of your final mosaic are shown.
13. To make a mosaic including different fields of interest without the external zones, go to the option CONVEX HULL.
14. The process is exactly the same as the BOUNDING GRID one.
15. The shape of the acquisition field is shown beside.
16. Click on START EXPERIMENT to start the acquisition.



ACQUIRE MULTIPLE STAGE POSITIONS

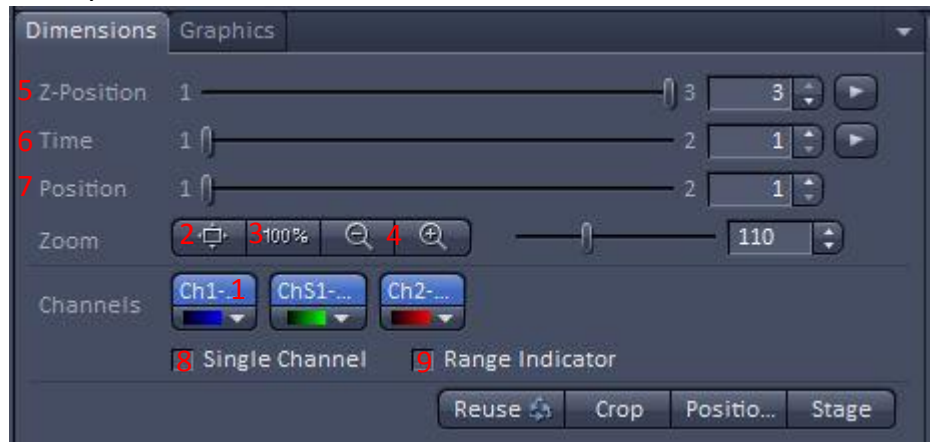
1. Proceed in the same way than in the previous section ACQUIRE ONE OR SEVERAL COLORS to fix the settings.
2. Tick POSITIONS to open the tool box in the menu MULTIDIMENSIONAL ACQUISITION.
3. Move to the interest position then click on ADD in the POSITION LIST tab.
4. Do the same for each position.
5. To acquire each well of a multiple-well plate, go to SAMPLE CARRIER.
6. Click on PROPERTIES and load parameters of your multiple-well plate.
7. Click on CALIBRATE to take these parameters into account.
8. Select wells of interest with the buttons SELECT, SELECT ALL or CLEAR ALL.
9. Tick OBJECTIVE LOWERED WHEN MOVING STAGE if you want to lower the objective for each displacement. (For oil objectives)
10. Click on START EXPERIMENT to start the acquisition.
11. Another solution to localize fields of interest is to acquire quickly a mosaic with a low resolution and spot wanted positions on the image by clicking on POSITIONS. Coordinates are saved in the POSITION LIST and you start the acquisition after having unticked TILE SCAN.



DISPLAYING ACQUIRED IMAGES

Under the image there is a board which allows you to select visible channels on the screen.

1. Tick the name (white on blue) of channels that you would like to display on the screen. Be careful to not untick all channels, otherwise the image is black.



2. Wide screen zoom

3. Native zoom (image pixel = screen pixel)

4. Zoom in / zoom out

5. Select z position

6. Select time position

7. See a position

8. Tick SINGLE CHANNEL to see channels separately.

9. Tick RANGE INDICATOR to see setting colors in order to avoid saturation.

10. See all channels superimposed.

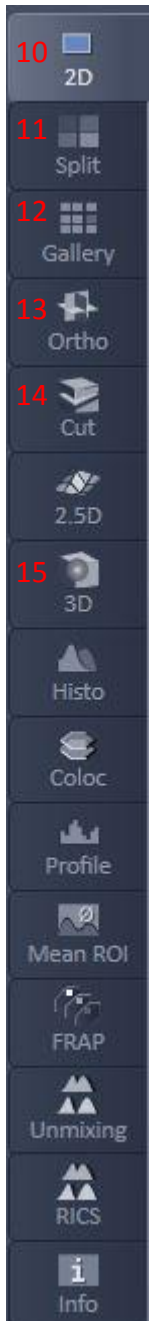
11. See all channels separately.

12. See all images gallery in function of time, z or wavelength

13. Orthogonal section XZ and YZ

14. Section in 3D

15. 3D representation

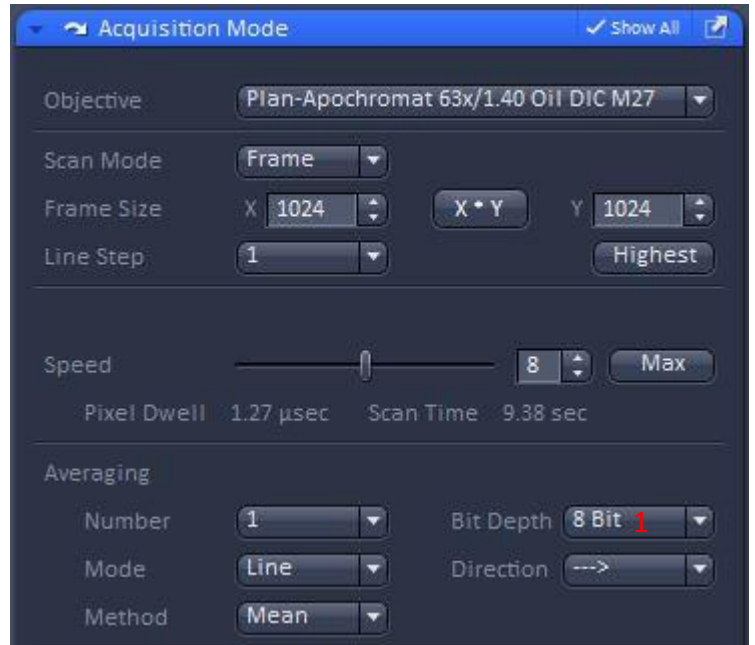


SIGNAL QUANTIFICATION

To quantify a fluorescence signal and if you want to compare those images, you have to acquire them with the same acquisition parameters (same laser power, same gain and Offset for PMTs).

If possible, acquisition conditions must be adjusted on the sample whose fluorescence signal is the brightest. In this way, the other samples won't be saturated.

1. For more precision in quantification measurement, images can be acquired in 12 bits. Go to the ACQUISITION MODE tool box and select BIT DEPTH 12bits.
2. Adjust acquisition conditions (Gain, Offset and laser power) on the brightest sample
3. Acquire all your samples without modifying your acquisition parameters
4. In case of one or several channels are saturated, you just need to adjust the PMT gain. Images could be corrected thanks to an acquisition of PMT range (intensity in function of tension).



For more details about images acquisition for quantification, don't hesitate to contact facility engineers.

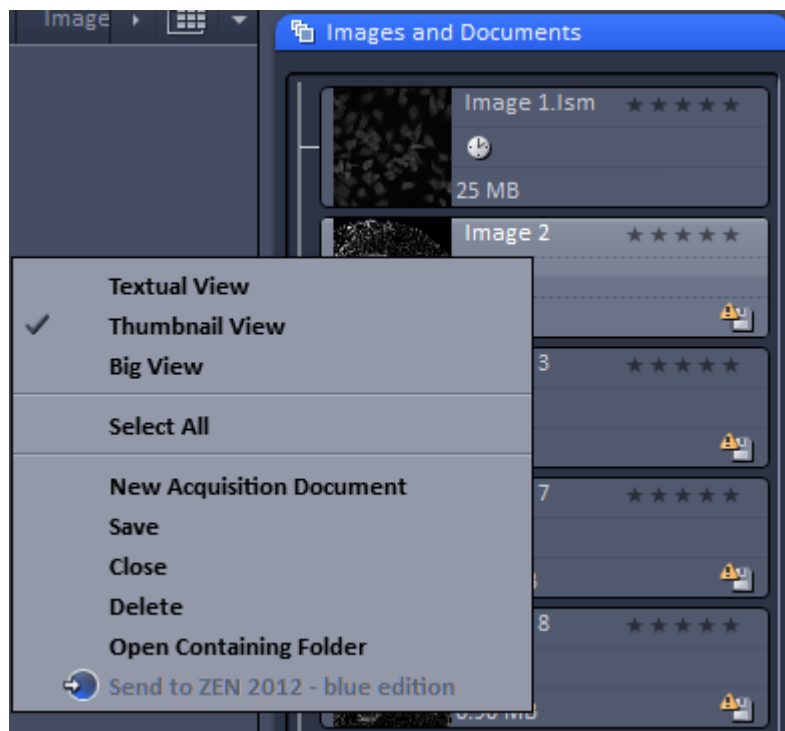
SAVING IMAGES

Select the image then save in .dzi

FILE – SAVE in the folder USER/year/month/day/user name.

You can select all acquired images with a right click on the blue edge then SELECT ALL and SAVE. You can also delete all of them by selecting DELETE.

You can select images of interest by clicking on while maintaining the Ctrl key.



SWITCH OFF THE SYSTEME

Warning !!!

If you used the temperature and CO₂ controllers, please untick all boxes and close the CO₂ bottle.

Check on the schedule online if the system is booked after your session.

If the system is booked after your session or in 4h after, you must not completely switch off, but proceed in this way :

1. Check that all your data are correctly saved.
2. Check that all the objectives are cleaned (lens + border).

If the system is not booked after you, you must completely switch off :

1. Check that all your data are correctly saved.
2. Close the ZEN software (menu *File* → *Exit*).
3. Switch off the computer (select in the tool bar *START* → *SHUTDOWN*).
4. On the small box, put the Argon laser knob at the minimum position.
5. On the small box, put the button down on « IDLE POWER ».
6. On the power box of Argon laser, turn the Power key on 0.
7. Check that all objectives are cleaned (lens + border).
8. Put the SYSTEM PC and COMPONENTS buttons on 0.
9. Switch off the fluorescence lamp.
10. When the Argon laser ventilation is off, put the MAIN SWITCH button on 0.

